Peters et al., http://www.jcb.org/cgi/content/full/jcb.201211146/DC1

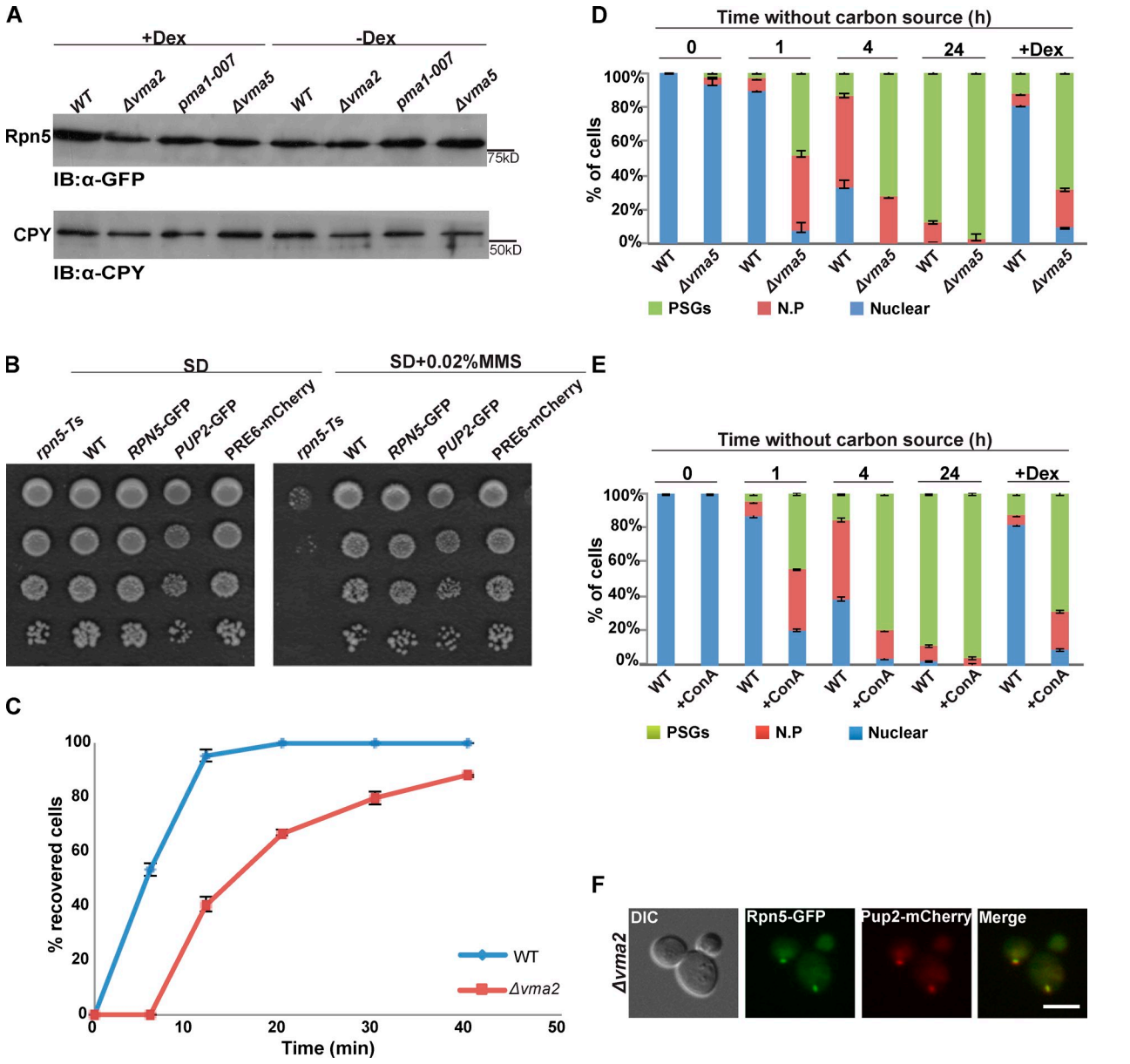

Figure S1. **Controls for the experiments described in Fig. 2.** (A) Proteasome levels are stable in the absence of dextrose in Δ*vma2*, pma1-007, and Δ*vma5* mutants' background. Protein extracts were prepared from logarithmic yeast strains carrying Rpn5-GFP in combination with the indicated mutants, and the wt control was grown in SD (+dextrose [+DEX]) or SC (−dextrose). Cell extracts were separated by SDS-PAGE and immunoblotted with the α-GFP antibody (immunoblot [IB]: αGFP) and α–carboxypeptidase Y (CPY), which was used as a loading control. The migration of the nearest protein molecular weight size marker (Precision Plus Protein standards, Dual Color; Bio-Rad Laboratories) for both Rpn5-GFP and carboxypeptidase Y is shown. (B) The expression of the fusion proteins Rpn5-GFP, Pup2-GFP, and Pre6-mCherry has no effect on proteasome activity. 10-fold serial dilutions of the indicated strains were spotted on SD medium lacking (control) or supplemented with 0.02% of the radiomimetic drug methyl methanesulfonate (MMS). Cells were incubated at 30°C for 3 d. The strain harboring the temperature-sensitive allele of the proteasomal lid subunit *RPN5 (rpn5-Ts)* and the wt were used as positive and negative controls. (C) The kinetics of Rpn5-GFP disassembly and relocalization back into the nucleus in response to glucose replenishment is significantly slower in Δ*vma2* mutants. Logarithmic wt and Δ*vma2* cells expressing the lid subunit Rpn5-GFP were washed and resuspended in glucose-free medium (t = 0) for 24 h and then transferred back into a glucose-rich medium (+dextrose). Cells were visualized by DIC and GFP at the indicated time points. The kinetics of reappearance of cells without PSGs in response to glucose replenishment is shown (percentage of recovered cells). For each time point, a minimum of 200 cells was counted ($n > 200$). (D and E) The kinetics of PSGs formation is affected in cells deleted in V-ATPase subunit *VMA5* (Δ*vma5*) and treated with the V-ATPase–specific inhibitor concanamycin A. Similar experimental design as described in Fig. 2 (A and B), this time in the Δ*vma5* genetic background (D) and in the presence 100 µM concanamycin A (ConA). (E) The graphs represent the percentage of cell categories and kinetic analysis at the indicated time points. (F) Different CP and RP subunits colocalize to the same cytoplasmic dots in Δ*vma2*. Logarithmic Δ*vma2* cells expressing the CP subunit Pup2 (tagged with mCherry) and the RP subunit Rpn5-GFP were grown in the absence of a carbon source; the localization of both proteins was determined after 5 h. Bar, 5 µm. N.P, nuclear periphery. Error bars show the standard deviation between two independent experiments.

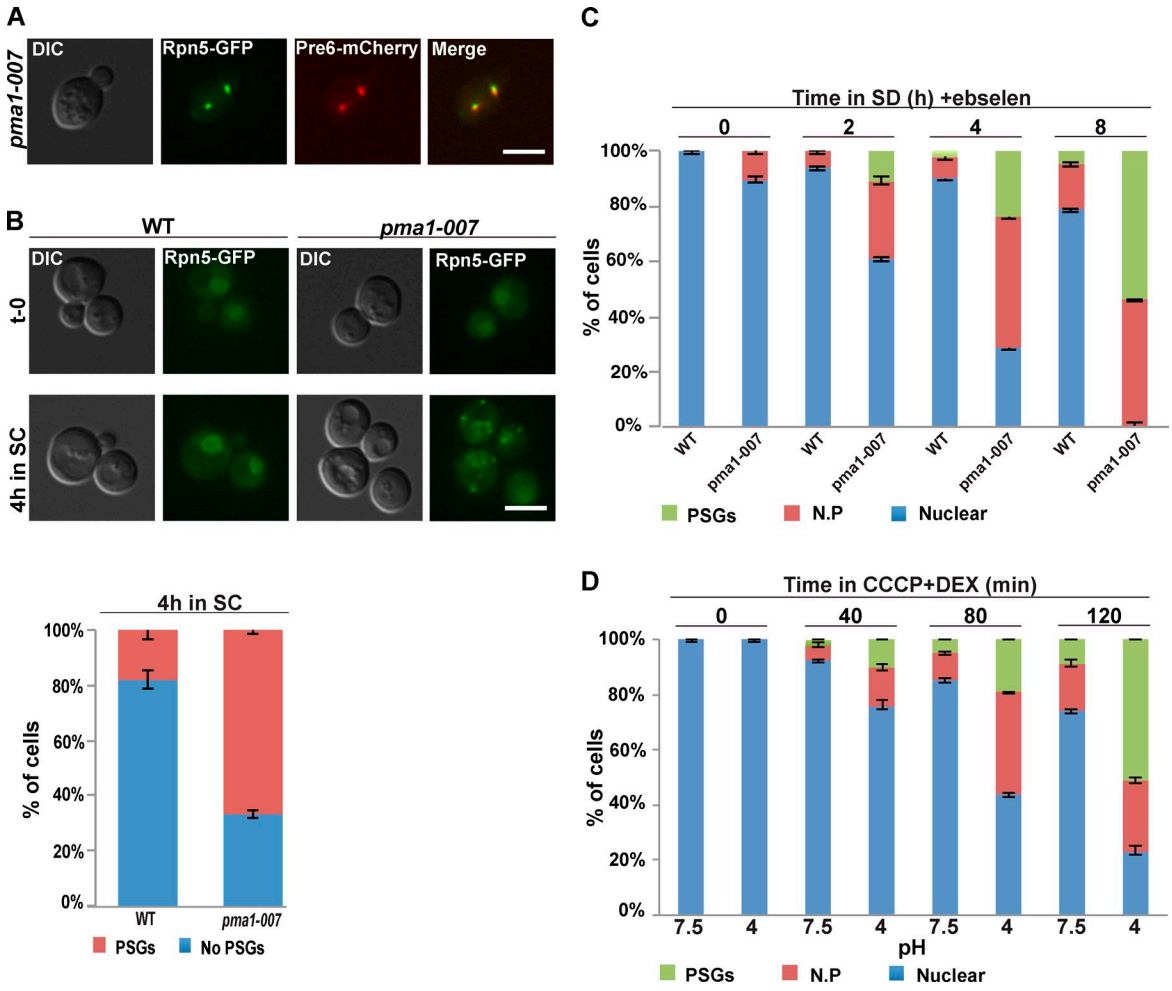

Figure S2. **Controls for the experiments described in Fig. 4 (A–C) and in Fig. 5 (D).** (A) Colocalization pattern of Rpn5-GFP and Pre6-mCherry in *pma1-007* cells grown in SD for 30 h. (B) The kinetics of PSG formation is faster in *pma1-007* mutants under low carbon source conditions. Logarithmically growing *pma1-007* and wt cells were grown in glucose-rich medium (t = 0), washed, and transferred to synthetic medium lacking carbon source for 4 h. Cells were visualized by DIC and GFP at the indicated time points. The representative images shown at t = 4 h show the dominant localization pattern that was quantitated in the graph. The localization of Rpn5-GFP was scored as the percentage of cells containing PSGs or cells showing nuclear/nuclear periphery GFP signal (no PSGs). (C) Similar to the experiment described in Fig. 4 B, this time the graph shows the quantitation of Rpn5-GFP localization in the presence of 100 µM ebselen, a drug that inhibits Pma1p. This drug was added both to wt cells and the *pma1-007* mutant. (D) Hos2 SPGs are formed as a result of low cytosolic pH. Logarithmically growing cultures (untreated) expressing Hos2-GFP were washed and resuspended in glucose-containing buffers, buffered to physiological pHi, pH 7.5 (control), and to pH 4.0, supplemented with CCCP. Cells were visualized by DIC and GFP at the indicated time points. The localization of Rpn5-GFP was scored as described in Fig. 5 E. N.P, nuclear periphery. Error bars show the standard deviation between two independent experiments. Bars, 5 µm.

Table S1. **Relevant genotype of strains used in this study**

Strains	Genotype	Source
Y7092	*MATα/can1Δ*::Ste1pr-*HIS5/lyp1Δ/ura3Δ0/leu2Δ0/his3Δ1/met15Δ0*	Boone laboratory
BY4741	*MATa/his3Δ/leu2Δ/met15Δ/ura3Δ*	Brachmann et al., 1998
BY4742	*MATα/ura3Δ0/leu2Δ0/his3Δ1/lys2Δ0*	Brachmann et al., 1998
YSB41	*RPN5*-GFP-*URA3*	Ben-Aroya et al., 2010
YSB48	*RPN5*-GFP-*URA3/Δpep4*::HygB/*can1*::Ste1pr-his5/*lyp1Δ*	This study
YSB91	*PRE6*-mCherry-HygB	This study
YSB383	*RPN5*-GFP-*URA3/pma1::007*-KanMX	This study
YSB230	*RPN5*-GFP-*URA3/Δvma2*::NatMX	This study
YSB441	*PUP2*-GFP-KamMX/*Δvma2*::NatMX	This study
YSB442	*PUP2*-GFP-KamMX	This study
YSB576	*RPN5*-GFP-*URA3/PRE6*-mCherry::HygB/*pma1::007*::KanMX	This study
YSB577	*RPN5*-GFP-*URA3/PUP2*::mCherry::HygB/*Δvma2*::NatMX	This study
YSB597	*HOS2*-GFP-*HIS3/Δvma2*::NatMX	This study
YSB598	*HOS2*-GFP-*HIS3/pma1::007*::KanMX	This study

References

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Brachmann, C.B., A. Davies, G.J. Cost, E. Caputo, J. Li, P. Hieter, and J.D. Boeke. 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*. 14:115–132. http://dx.doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2